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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Douglas P. Cerretti — Attorney Docket No.: 2517-D

Serial No.: 10/664,456 — Group Art Unit: 1656

Filed: September 19, 2003 — Examiner: William W. Moore

For: METALLOPROTEINASE-DISINTEGRIN POLYPEPTIDES

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, hereby declare that:

1. I am the same Douglas P. Cerretti named as an inventor in USSN 10/664,456.

2. I have read the above-referenced patent application and I am familiar with the invention described in Claims 1-11, 14-23 and 26 now under consideration in that application.

3. I am aware that in an Office Action dated March 10, 2006, Claims 1-11, 14-23 and 26 were rejected in part because of concerns related to utility.

4. The following study was carried out under my supervision.

Expression cassettes encoding an Ig Kappa leader sequence, ADAM disintegrin domain, and C-terminal Fc region were constructed in bacterial plasmids then transferred into eukaryotic expression vectors (pDC409, EMBO J. 10:2821, 1991, or another mammalian expression vector). In addition to the disintegrin domain, this construct encodes additional portions of the extracellular portion of the ADAM (e.g., cysteine-rich region and EGF-like domain).

The expression vectors were transfected into COS-1, CV-1/EBNA, or 293/EBNA cells. Two days after transfection the cells were ^{35}S labeled for four hours. Supernatants and total cell lysates were prepared and aliquots were immunoprecipitated using protein A-sepharose beads to capture the Fc tagged polypeptides. ^{35}S labeled ADAM disintegrin-Fc polypeptides were run on 8-16% reducing gels and detected via autoradiography.

The cell type that produced the most soluble protein in the supernatant was used in a large scale (T-175 format, 20 flasks) transient transfection, and approximately one liter of supernatant was harvested after one week. ADAM disintegrin-Fc polypeptides were purified from the supernatants using affinity chromatography (protein A column). The polypeptides were characterized by determining the N-terminal amino acid sequence, amino acid composition, and protein integrity (SDS-PAGE under reducing and non-reducing conditions) before the polypeptides were used in FACS, immunoprecipitations, and biological assays.

Binding to endothelial cells

Primary human umbilical vein endothelial cells (HUVEC) were maintained in supplemented endothelial growth medium (EGM-2, Cambrex Bio Science Corporation, Walkersville, MD). HUVEC cultured under standard conditions were detached from their culture flask using a trypsin/EDTA solution (Cambrex Bio Science) following a rinse of the cell monolayer with pyrogen-free PBS (Gibco). A cell suspension was created by washing the detached cells with PBS containing 2% fetal bovine serum, 10% normal goat serum, 10% normal rabbit serum, 0.05% sodium azide, and 2 mM Ca^{2+} and Mg^{2+} (integrin-binding FACs block). The cells were pelleted by centrifugation at 4°C and 1800 rpm for three minutes. The cell pellet was then resuspended in FACs block at three million cells per milliliter (ml) and 0.1 ml of the suspension was added to polystyrene sample tubes (Falcon 2054).

The primary binding incubation step consisted of the addition of ADAM 29dis-Fc (Amgen Inc.), or human Tek delta Fc (Amgen Inc.) as a control, directly to the samples at a final concentration of 20 ug/ml. This primary incubation was incubated for 40 minutes with the tubes partially suspended in a water bath at 37°C.

The samples were then subjected to a wash, to remove unbound ADAM 29, with a 20-volume excess of FACs block and centrifuged as indicated above. The cell pellet was resuspended with 0.1 ml FACs block. A second incubation step was then carried out by adding a goat anti-human IgG Fc-specific PE (Jackson Immunochemicals), at a final dilution of 1:100. This incubation was carried out for 40 minutes in a water bath at 37°C.

The samples were washed, to remove unbound anti-human Fc-PE, with FACs block, centrifuged, and resuspended in 0.3 ml FACs block. Detection of ADAM 29 binding to HUVEC was performed by reading the samples on a FACSCalibur (Becton Dickinson). Determination of ADAM 29 binding to HUVEC was made by noting the increased mean fluorescent intensity (MFI) of the ADAM 29 sample over the control Fc sample.

The presence of ADAM 29dis-Fc binding to the surface of HUVECs is directly proportional to the increased MFI displayed for that sample over the control sample (figure 1). The integrin "activation" conditions brought about by incubation in the presence of divalent cations, that is Ca^{2+} and Mg^{2+}, lends itself to the reasonable expectation that ADAM 29dis-Fc is binding to one, or more, integrins on the surface of HUVECs.

Figure 1. Flow cytometric results of ADAM 29dis-Fc binding to HUVEC

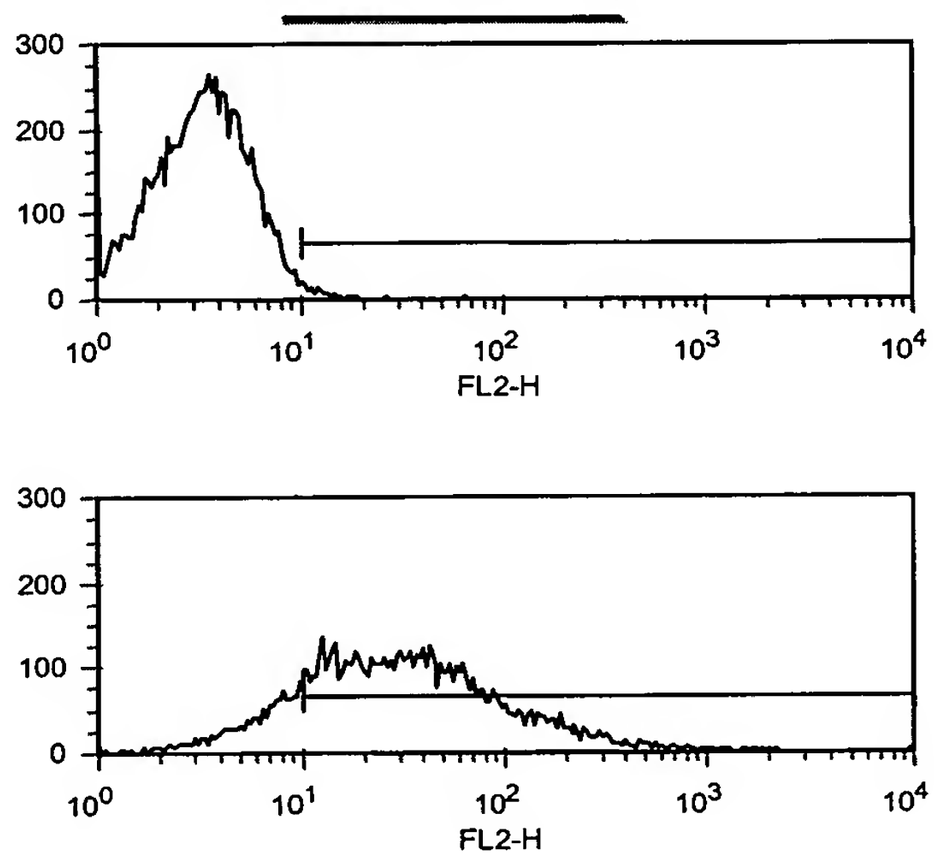

Figure 1, top panel. Negative binding of control, human Tek delta Fc, to the surface of HUVEC with an MFI of 3.3.

Bottom panel. Binding of human ADAM 29dis-Fc to the surface of HUVEC with an MFI of 30.2. This MFI represents relatively strong binding of ADAM 29 to HUVEC when compared to the control Fc.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6/2/06 — Douglas P. Cerretti

Date — Douglas P. Cerretti

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